Remarks

Reconsideration and withdrawal of the rejections set forth in the Office Action dated April 1, 2002 are respectfully requested.

I. Rejections under 35 U.S.C. §103

Claims 1-30 were rejected under 35 U.S.C. §103 as allegedly obvious over Martin *et al.* (U.S. Patent No. 5,213,804) in view of Mori *et al.* (*Cancer Chemother. Pharmacol.*, 35:447 (1995)).

Claims 1-30 were rejected under 35 U.S.C. §103 as allegedly obvious over Martin *et al.* in view of Mori *et al.* further in view of Kassis *et al.* (U.S. Patent No. 5,077,034).

These rejections are respectfully traversed for the following reasons.

Summaries of the present invention and of the cited documents are provided in Applicants' response submitted December 26, 2001.

A. Analysis of Examiner's Rejections

The Examiner finds Applicants' arguments presented in the December 26, 2001 response unpersuasive for four reasons. Each reason, and Applicants' rebuttal thereto, is given below.

1.0 Examiner's First Argument

The Examiner finds Applicants' argument that modification of Martin *et al.* to include the lipid prodrug of Mori *et al.* would compromise the desired extended blood circulation lifetime of the Martin *et al.* liposomes speculative for want of evidence.

1.1 Applicants' Rebuttal to the First Argument

Applicants' assertion that ligands attached to the surface of PEG-coated liposomes compromise the extended blood-circulation lifetime achieved by the

presence of PEG chains is well documented in the literature[1]. For example, Torchilin *et al.*[1] conducted a study comparing the circulation time and binding avidity of PEGylated, long-circulating liposomes with and without surface-attached antibodies. Torchilin *et al.* reported that liposomes coated with 4% PEG and having no antibodies had a blood circulation lifetime in rabbits of approximately 300 minutes. The same liposomes with surface-attached antibodies had a reduced blood circulation lifetime of 200 minutes. A copy of the Torchilin *et al.* paper is enclosed, and the noted data is set forth on page 2718 and is highlighted for the Examiner's convenience.

Thus, Applicants' argument is not mere speculation. Applicants maintain that there is no motivation to modify Martin *et al.* to include the prodrug of Mori *et al.* Martin *et al.* are concerned with extending the blood circulation lifetime of liposomes to achieve localization in a solid tumor. The extended blood circulation is achieved by coating the liposomes with PEG. Placing a drug, such as dpFUdR, on the surface of the PEGylated liposomes of Martin *et al.* would be expected to have an effect similar to that of the surface-attached antibodies in the Torchilin *et al.* study. A person of skill based on the Torchilin *et al.* study would reasonably expect surface-attached moieties to render PEG-coated liposome more susceptible to RES uptake than PEG-coated lipsomes lacking the surface-attached moiety.

Thus, the Examiner is respectfully requested to reconsider his first reason for finding Applicants' arguments unpersuasive.

2.0 Examiner's Second Argument

Second, the Examiner asserts that the purpose of PEG chains on a liposome is to mask factors that cause RES recognition of the liposomes, and that PEG would mask a surface-attached radiosensitizer. The Examiner states that (1) the purpose of PEG is to mask factors, such a liposome size, charge, degree of unsaturation of lipids, surface moieties, from RES recognition and (2) "it is logical that PEG containing repeating units of ethylene glycol will extend further on the liposomal surface than the deoxyuridine

[1]Torchilin *et al., The FASEB Journal*, 6:2716 (1992).

compound." Thus, the Examiner disagrees that the liposome composition of Martin *et al.* is compromised by the inclusion of a radiosensitizer.

2.1 Applicants' Rebuttal to the Second Argument

Indeed, the purpose of PEG is to mask surface moieties from RES recognition. However, the ability of PEG to mask surface-attached moieties is not absolute. Numerous studies[2,3,4] (copies enclosed) have looked at the issue of balancing, on one hand, the need to mask a surface-attached moiety to maintain a long blood-circulation lifetime and, on the other hand, the need to leave the surface-attached moiety accessible for action. That is, there must be sufficient PEG coating the surface of the liposome to mask the surface features yet not so much PEG that activity of the surface ligand is prohibited. Sufficiency of PEG coating is a function of both PEG concentration and molecular weight, where a low molecular weight PEG requires a higher concentration to coat a liposome to the same degree as a higher molecular weight PEG[2,4].

The paper by Noppl-Simson and Needham[2] (copy enclosed) illustrates these points. Noppl-Simson and Needham prepared PEG-coated liposomes having surface attached biotin. They found that 10 mol% PEG having a molecular weight of 750 Daltons almost completely blocked binding of avidin to the surface-attached biotin. Using a larger 2000 molecular weight PEG the same result was achieved at a concentration of only 4 mol% (see page 1395, paragraph bridging Cols. 1-2). Hristova and Needham[4] state that "increasing the concentration of grafted polymer, as well as the molecular weight of the polymer, further improves the repulsive properties of the lipid bilayer surfaces, creating a denser, larger, brush" (see page 44, highlighted portion). Klibanov *et al.*[3] report that "PEG-PE incorporation of immunoliposomes resulted in a low level of target binding (see abstract and page 146).

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2 Noppl-Simson and Needham, *Biophysical J.*, 70:1391 (1996)

3 Klibanov, A. *et al., Biochim. Biophys. Acta*, 1062:142 (1991).

4 Hristova and Needham, STEALTH LIPOSOMES, CH. 5, Lasic and Martin et al., Eds., CRC Press, 1995.

Thus, the Examiner is correct in stating that "PEG containing repeating units of ethylene glycol will extend further on the liposomal surface than the deoxyuridine compound'; however, this fact does not speak to whether or not a surface-attached compound will or will not compromise the liposome's blood circulation lifetime. The noted studies all indicate that surface masking is a function of PEG molecular weight (chain length) and concentration; and that a low molecular weight PEG at a low concentration will not mask surface-attached moieties.

The Examiner's reasoning implies, and indeed the argument assumes, that PEG "extension from the liposome surface" is the critical feature in providing a long-circulating liposome. As supported by the noted references, this is not the case; since liposomes with a low concentration of PEG (either of low or high molecular weight, but particularly a low molecular weight) may not adequately mask surface-attached moieties to maintain blood circulation lifetime.

Thus, Applicants' argument that the presence of dpFUdR on the surface of Martin et al.'s PEGylated liposomes would reasonably be expected to compromise blood-circulation time is well supported in the literature. The Examiner is respectfully requested to reconsider his reasoning.

3.0 Examiner's Third Argument

Third, the Examiner disagrees with Applicants' argument that since the dpFUdR liposomes of Mori *et al.* have a decreased therapeutic index, one would not be motivated to incorporate dpFUdR into PEGylated liposomes. The Examiner's basis for disagreeing is that the reduced therapeutic index of dpFUdR liposomes is due to localization of the liposomes in the liver and subsequent release from the liver (as disclosed by Mori *et al.*, page 455, Col. 1, line 3 et seq). The Examiner believes that this fact would motivate one to include PEG in dpFUdR liposomes to avoid liver uptake.

3.1 Applicants' Rebuttal to the Third Argument

First, this argument relies on the assumption that the presence of dpFUdR on the liposome surface has no influence on the RES-masking ability of the PEG coating. This assumption has been disproven in points 1.1 and 2.1 above.

Second, Applicants take issue with the Examiner's reading page 455, Col. 1, line 3 et seq. of Mori *et al.* The Examiner reads Mori *et al.* as teaching that an increased toxicity (and thus a reduced therapeutic index) is due to localization of the prodrug in the liver macrophages and subsequent release from those cells of the parent drug FudR into the blood. The Examiner uses this reading to find motivation to include PEG on the surface of Mori *et al.* liposomes, since a PEG coating masks liposomes from liver uptake.

The sentence on page 455 at line 3 to line 8 refers to studies using antibody-free, non-PEG-coated ("conventional") liposomes, as clearly stated by Mori *et al.* on lines 8-13 of Col. 1, page 455:

> The increased toxicity of dpFUdR incorporated into liposomes was believed to be due to the predominant localization of the prodrug in the liver macrophages and the subsequent release from those cells of the parent drug FudR into the blood as a result of intracellular hydrolysis of dpFUdR. In these studies, however, we used conventional liposomes, i.e., antibody-free liposomes, as a carrier of dpFUdR and, thus, the majority of the delivered liposomal dpFUdR accumulated in the liver due to the high affinty of conventional liposomes for this organ.

In this passage, Mori *et al.* teach that liposomes with no antibody and no PEG but with dpFUdR have increased toxicity due to liver uptake. Mori *et al.* in the next paragraph go on to teach that including targeting antibodies on the lipsomes might be expected to reduce liver accumulation and improve the toxicity problem:

> Since 34A-liposomes with a reduced affinity to the RES are shown to deliver drugs to the lung and to reduce drug accumulation in the liver macrophages, the use of 34A liposomes as carriers for dpFUdR is expected to lead to an improved therapeutic index of the drug together with reduced toxicity in the therapy of lung tumors. .....More experiments are needed to assess the toxicity of immunotargeted liposomal dpFUdR to the lung. (Mori *et al.*, page 455, lines 14-29).

Based on this, Applicants would argue exactly the opposite of what the Examiner argues. That is, Applicants assert that there is no motivation to put PEG on the liposomes of Mori *et al.*, since to do so would mask the surface-attached antibodies which Mori *et al.* teach are likely to reduce toxicity.

In the event the Examiner finds this argument that PEG chains mask surface-attached antibodies speculative, Applicants point the Examiner to the previously discussed Noppl-Simson[2] paper (teaching that PEG blocks binding of avidin to liposome surface-attached biotin) and Klibanov *et al.*[3] (reporting that "PEG-PE incorporation of immunoliposomes resulted in a low level of target binding") and additionally to Maruyama *et al.*[5], enclosed herewith (see highlighted text on pages 75 and 79; teaching "surface PEG polymers hinder binding of liposome-bound antibodies with target antigens).

Thus, since it is known in the art that the presence of PEG chains masks surface-attached antibodies and reduces antibody binding affinity for its target, Applicants maintain the position that there is no motivation to add PEG to the liposomes of *Mori et al.*, since to do so would mask the surface-attached antibody needed for targeting to the lung, for both site specific delivery and for toxicity reduction. The Examiner is respectfully requested to reconsider his reasoning.

4.0 Examiner's Fourth Argument

Fourth, the Examiner disagrees with the Applicants' assertion that one of skill would not be motivated to PEGylate the dpFUdR, antibody-targeted liposomes of *Mori et al.* since PEG would mask the antibody. The Examiner argues that one would want to PEGylate the liposomes of *Mori et al.* in order to decrease uptake by the liver, thereby improving the therapeutic index. The Examiner also asserts that Applicants' position is mere speculation.

4.1. Applicants' Rebuttal to the Fourth Argument

This point has been adequately addressed in 3.1 above and any assertion that PEG renders the activity of surface-attached moieties as being speculative is thoroughly discredited by the references[2,3,4,5] submitted herewith.

5.0 Summary

The combined teachings of Martin *et al.* and Mori *et al.* are cited against the present claims. Martin *et al.* teach the addition of PEG to liposomes to extend their blood circulation lifetime. Mori *et al.* teach liposomes having surface-attached antibodies and dpFUdR.

The Examiner asserts that it would be obvious to modify the liposomes of Martin *et al.* to include dpFUdR "because of its effectiveness shown by Mori *et al.*." (Office action dated April 1, 2002, page 3, lines 5-6).

Applicants' disagree. The effectiveness of the liposomes of Mori *et al.* is dependent on the antibody targeting, as discussed above in point 3.1. Thus, as a whole Mori *et al.* teach that antibody-targeted, dpFUdR liposomes are effective. To suggest that Mori *et al.* simply teaches that dpFUdR liposomes are effective is an oversimplification, since antibodies are clearly needed for the effectiveness (see point 3.1 above). There is no basis for selecting from the teaching of Mori *et al.* only the surface-attached dpFUdR at the exclusion of the surface-attached antibodies. It is

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[5] Maruyama, K. *et al., Biochim. . Biophys. Acta*, 1234:74 (1995).

improper to pick and choose the elements of a teaching to reconstruct Applicants' invention.

The Examiner also asserts that it would also have been obvious to use the PEG-lipid of Martin *et al.* in the antibody-targeted, duFUdR liposomes of *Mori et al.*, since the PEG would increase the circulation time of Mori *et al.*'s liposomes.

This argument fails, since such a modification of Mori *et al.* would mask the antibodies (as extensively discussed in points 1.1, 2.1, and 3.1 above and supported by the enclosed references) and either render Mori *et al.* inoperable or impermissibly change the principle of operation (MPEP 2143.01).

Thus, Applicants maintain that there is no motivation to combine the teachings of Martin *et al.* and Mori *et al.*, and request withdrawal of the rejection.

B. Rejection Based on Martin *et al.* in view of Mori *et al.*, or vice versa, and further in view of Kassis.

The disclosure of Kassis is cited to provide a teaching of other radiosensitizers not discussed by Mori *et al.* All of the arguments above apply to this rejection and withdrawal is respectfully requested.

III. Conclusion

In view of the above remarks, Applicants submit that the claims pending in the application are in condition for allowance. A Notice of Allowance is therefore respectfully requested.

If in the opinion of the Examiner, a telephone conference would expedite prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4402.

Respectfully submitted,

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